Please replace the paragraph beginning on page 3, line 28, with the amended paragraph as follows:

By "activates protein kinase Bα" we include the meaning that upon phosphorylation by the said 3-phosphoinositide-dependent kinase the activity of protein kinase Bα to a given substrate increases by at least ten-fold compared to the protein kinase Bα which has not been so phosphorylated, preferably by at least 20-fold and more preferably by at least 30-fold. Suitably, the activity of protein kinase Bα is measure using the synthetic peptide RPRAATF (SEQ ID NO:9).

Please replace the paragraph beginning on page 6, line 28 with the amended paragraph as follows:

A particularly preferred embodiment is a substantially pure 3-phosphoinositide-dependent protein kinase that phosphorylates and activates protein kinase Bα the 3-phosphoinositide-dependent protein kinase comprising the amino acid sequences ANSFVGTAQYVSPELL (SEQ ID NO:3) or AGNEYLIFQK (SEQ ID NO:4) or LDHPFFVK (SEQ ID NO:5) or two or more of these sequences or amino sequences with from 1 to 4 conservative substitutions thereof. What is meant by "conservative substitutions" is described below.

Please replace the paragraph on page 7, lines 6-7 with the amended paragraph as follows:

A particularly preferred embodiment is a polypeptide which comprises the amino acid sequence (SEQ ID NO:1)

Please replace the paragraph on page 9, lines 8-9 with the amended paragraph as follows:

A particularly preferred embodiment of the invention is a polynucleotide comprising the nucleotide sequence (SEQ ID NO:2)

Please replace the paragraph beginning on page 22, line 5 with the amended paragraph as follows:

In a preferred embodiment the antibody is raised using any one of the peptide sequences ANSFVGTAQYVSPELL (SEQ ID NO:3) or AGNEYLIFQK (SEQ ID NO:4) or LDHPFFVK (SEQ ID NO:5). It is preferred if polyclonal antipeptide antibodies are made. Other peptides may be used to make antibodies, for example the peptides RQRYQSHPDAAVQ (SEQ ID NO:6) and LSPESKQARANS (SEQ ID NO:7).

Please replace the paragraph beginning on page 31, line 26 with the amended paragraph as follows:

Figure 1. SDS Polyacrylamide gel of purified GST-PKBα. 293 cells were transiently transfected with the pEBG2T DNA construct expressing GST-PKBα, serum starved for 16 h and, after cell lysis, GST-PKBα was purified by affinity chromatography on ~~glutathione-Sepharose~~ glutathione-SEPHAROSE™ (see Methods). ~~"Sepharose"~~ SEPHAROSE™ is a trade mark. The ~~glutathione-Sepharose~~ glutathione-SEPHAROSE™ eluate (3 µg protein was electrophoresed on a 10% SDS polyacrylamide gel and stained with ~~Coomassie~~ COOMASSIE™ blue. ~~"Coomassie"~~ COOMASSIE™ is a trade mark. The position of the molecular mass markers, glycogen phosphorylase (97 kDa), bovine serum albumin (67 kDa) and ovalvumin (43 kDa) are indicated.

Please replace the paragraph on page 32, lines 6-17 with the amended paragraph as follows:

Figure 2. Identification and purification of a

PtdIns(3,4,5)P3 dependent protein kinase (PDK1) which activates GST-PKBα. Following the PEG precipitation PDK1 was chromatographed sequentially on a SP-~~Sepharose~~ SEPHAROSE™ (A) and heparin-~~Sepharose"~~ SEPHAROSE™ (B) and Peak 2 from the latter column was chromatographed on Mono ~~S~~ S™(C)(see Methods). "Mono ~~S~~" S™ is a trade mark. PDK1 activity was assayed within in the presence of phospholipid vesicles comprising 100 μM PtdCho, 100 μM PtdSer, 10 μM D-enantiomer of *sn*-1-stearoyl2-arachidonyl PtdIns(3,4,5)P3(open circles) or vesicles comprising only 100 μM PtdCho and 100 μM PtdSer (closed circles). The broken lines indicate the salt gradient and the solid line the absorbance at 280nm. The amount of protein eluted from the Mono ~~S~~ S™ column was too low to see any absorbance at 280 nm.

Please replace the two paragraphs on page 35, lines 28-30 with the amended paragraphs as follows:

Figure 8. Details of IMAGE Consortium clone ID 526583 (SEQ ID NO:27)

Figure 9. Details of IMAGE Consortium clone ID 626511 (SEQ ID NO:28)

Please replace the paragraph on page 36, lines 1-2 with the amended paragraph as follows:

Figure 10. Nucleotide sequence coding for, and deduced amino acid sequence of, PKD1 (SEQ ID NO:2 and SEQ ID NO:1, respectively).

Please replace the paragraph on page 36, lines 4-9 with the amended paragraph as follows:

Figure 11. Alignment of the amino acid sequences of human PDK1 and *Drosophilia* DSTPK61 (SEQ ID NO:29). The alignment

was carried out using the Clustal W program [46]. Asterisks indicate identities between PDK1 and STK61. The catalytic domain comprises residues 83-342 of PDK1 and residues 165-487 of STPK61. The putative PH domains lie between residues 450 and 550 of PDK1 and 581 and 684 of STPK61B.

Please replace the paragraph on page 45, lines 19-25 with the amended paragraph as follows:

Assay of PDK1. The assay was carried out in two stages; in the first GST-PKBα was incubated with PDK1 in the presence of MgATP and phospholipid vesicles to permit activation of GST-PKBα. In the second stage, the solution was made 0.5% (by vol) in Triton X100 (which completely inhibits phosphorylation and activation of GST-PKBα without affecting GST-PKBα activity, see Fig 3B), together with Mg[γ32P]ATP, and the specific PKBα substtate peptide RPRTAAF (SEQ ID NO:9) [16].

Please replace the paragraph on page 56, lines 6-14 with the amended paragraph as follows:

LDHPFFVK (SEQ ID NO:5);
ANSFVGTAQYVSPELL (SEQ ID NO:3);
AGNEYLIFQK (SEQ ID NO:4);
AHPFFESVTWENLHQQTPPK (SEQ ID NO:11);
SGSNIEQYIHDLDSNSFELDL (SEQ ID NO:12);
QAGGNPWHQFVENNLILK (SEQ ID NO:13);
QLLLTEGPHLYYVDPVNK (SEQ ID NO:14);
TFFVHTPNR (SEQ ID NO:15);
YQSHPDAAVQ (SEQ ID NO:16);

Please replace the paragraph beginning on page 56, line 30 with the amended paragraph as follows:

Most of the open reading frame of PDK1 was derived by interogation of the dbest database of the National Centre for Biological Information. A full length cDNA clone for PDK1 was isolated by hybridisation screening of a cDNA library, in the vector λZAP, made from the human breast cancer cell line MCF7 (a gift of P. Mitchell, Institute of Cancer Research, Sutton, UK). The PDK1 probe for the screening was generated by RT-PCR with the primers CTGAGCCAGTTTGGCTGC (SEQ ID NO:17) and ACGTCCTGTTAGGCGTGTGG (SEQ ID NO:18) corresponding to nucleotide 1138-1567 of the PDK1 sequence, with MCF cDNA as template. DNA sequencing was carried out on an Applied Biosystems 373 DNA automatic sequencer using the Taq dye terminator cycle sequencing kit.

Please replace the paragraph on page 59, lines 13-15 with the amended paragraph as follows:

1. ANSFVGTAQYVSPELL (SEQ ID NO:3)
2. AGNEYLIFQK (SEQ ID NO:4)
3. LDHPFFVK (SEQ ID NO:5)

Please replace the paragraph on page 59, line 28 with the amended paragraph as follows:

5'TTTGT(G/T)GGIACIGCICA(A/G)TA(T/C)GT 3' (SEQ ID NO:19)

Please replace the paragraph on page 60, line 1 with the amended paragraph as follows:

5'TTIAC(A/G)AA(A/G)AAIGG(A/G)TG(A/G)TC 3' (SEQ ID NO:20)

Please replace the paragraph on page 60, line 11 with the amended paragraph as follows:

5'AA(T/C)GA(A/G)TA(T/C)(C/T)TIAT(T/C/A)TT(TC)CA(A/G)AA 3' (SEQ

ID NO:21)

Please replace the paragraph beginning on page 62, line 15 with the amended paragraph as follows:

Preparation of DNA expression constructs encoding GST-PDK1, Myc-PDK1. Two overlapping human ESTs encoding PDK1 (GenBank accession numbers AA121994 and AA186323 corresponding to nucleotides 98 to 708 and 467 to 1811 of PDK1 (Fig 1) were obtained from the I.M.A.G.E. consortium [42] and sequenced. The two sequences were joined together by a *Sca*I restriction enzyme digest of each EST clone, and the appropriate restriction fragments obtained from these digests were ligated to generate a plasmid containing a continuous PDK1 sequence from nucleotides 154-1670. This construct was used as a template for a PCR reaction to generate a N-terminal epitope-tagged Myc-PDK1 (amino acid residues 52-556) construct. This was achieved using the oligonuclotides GCGGAGATCTGCCACCATGGAGCAGAAGCTGATCTCTGAAGAGGACTTGGACGGCACTGCAG CCGAGCCTCGG (SEQ ID NO:22) and GCGGGGTACCTCACTGCACAGCGGCGTCCGGGTG (SEQ ID NO:23) that incorporate a *Bgl*II site (underlined) and a *Kpn*I site (double underlined). The resulting PCR fragment was subcloned into the *Bgl*II/*Kpn*I sites of an pSP72 cloning vector, and the nucleotide sequence confirmed by DNA sequencing. The Myc-PDK1 coding sequence was subcloned from this vector as a *Bgl*II-*Kpn*I fragment into the *Bam*HI and *Kpn*I sites of the eukaryotic expression vectors pEBG2T [43] in order to generate a construct for the expression of GST-PDK12 in 293 cells. The same fragment was ligated into the *Bgl*II and *Sal*l sites of the vector pCMV5 [44] to generate a construct for the expression of Myc-PDK1 in 293 cells. The structure of each construct was verified by DNA sequencing, and plasmid SNA for transfection was purified using the Qiagen plasmid Mega kit according to

the manufacturer's protocol.

Please replace the paragraph beginning on page 63, line 12 with the amended paragraph as follows:

N-terminal Myc epitope ragged PDK1 constructs encoding the full length protein (residues 1 to 556) and lacking the PH domain (residues 1 to 450) were generated by a PCR approach using the full length full length PDK1 cDNA isolated from the MCF7 library. This was achieved by using the 5' primer gcggagatctgccaccatggagcagaagctgatctctgaagaggacttggccaggaccacca gccagc tgtatgacg (SEQ ID NO:24) (for both the full length and ΔPH-PDK1 constructs) and the 3' primers gcggggtacctcactgcacagcggcgtccgggtg (SEQ ID NO:23) (for full length PDK1) and gcggggtacctcagtgccaagggtttccgccagcctgctt (SEQ ID NO:25) (for the ΔPH-PDK1 construct). The resulting PCR fragments were cloned into the pCR 2.1-TOPO vector and subsequently subcloned into the pEGB-2T vector as *BglII-KpnI* fragment and pCMV5 as an *EcoRI-KpnI* fragment. A full length catalytically inactive PDK1 construct in which Asp223 was changed to Ala was created by the PCR-based megaprimer strategy (Tao & Lee (1994) In: Griffin & Griffin (eds) PCR Technology: Current Innovations, CRC Press, Boca Raton, Florida, pp 69-83) and then subcloned into pCMV5 and pEBG-2T as described above. The structure of each construct was verified by DNA sequencing, and plasmid DNA for transfection was purified using the Qiagen plasmid Mega kit according to the manufacturer's protocol.

Please replace the paragraph at page 64, lines 8-17 with the amended paragraph as follows:

Preparation of DNA expression constructs encoding GST-APH-PKBα. A PCR based strategy was used to prepare a GST-ΔPH-PKBα (encoding residues 118-479 PKBα) construct using as a template

a full length human PKBα construct that was isolated from a human skeletal muscle cDNA library and subcloned into the ~~pBluescriptSK~~ pBLUESCRIPTSK™ vector. The GST-ΔPH-PKBα construct was obtained using the 5' primer cgggatccatggacttccggtcgggctca (SEQ ID NO:26) and 3' primer was the T7 oligonucleotide of ~~pBluescriptSK~~ pBLUESCRIPTSK™ vector. The resulting PCR fragment was cloned into ~~pBluescriptSK~~ pBLUESCRIPTSK™ as a *BamHI-KpnI* fragment, and subsequently into pEBG- 2T as a *BamH1-KpaI* fragment.

Please replace the paragraph beginning at page 65, line 22 with the amended paragraph as follows:

Assay of GST-PDK1 and GST-DSTPK61 activity. GST-PDK1 and GST-DSTPK61 were diluted appropriately in Buffer B containing 1 mg/ml bovine serum albumin, and assayed for their ability to activate and phosphorylate GST-PKBα 21]. One unit of PDK1 activity was defined as that amount required to increase the basal activity of GST-PKBα by 1 unit of activity in one min. One unit of GST-PKBα activity was that amount of enzyme required to catalyse the phosphorylation of 1 nmol of the peptide RPRAATF (SEQ ID NO:9) in 1 min in an assay containing 0.1 mM RPRAATF (SEQ ID NO:9) [45]). In order to ensure that the assay was linear with respect to time the concentration of GST-PDK1 or GST-DSTPK61 was below 2 U/ml. At this concentration the level of phosphorylation of PBKα was <0.4 mol phosphate per mol protein in the 30 min assay.

Please replace the paragraph at page 66, lines 5-14 with the amended paragraph as follows:

Transfection of 293 cells with HA-tagged PBKα and Myc-PDK1. Human embryonic kidney 293 cells were cultured on 10 cm diameter dishes and transfected with 2 μg/ml pCMV5 DNA constructs encoding for HA-PKBα or HA-PKBα plus Myc-PDK1 [20].

After 24 h the cells were deprived of serum for a further 16 h and then stimulated for 10 min with either 100 ng/ml IGF1 or Buffer. The cells were lysed in 1.0 ml of ice-cold Buffer A, the lysate centrifuged at 4□C for 10 min at 13,000 x g and HA-PKBα immunoprecipitated from aliquots of the supernatant (10 µg protein) [20] and assayed for PKBµ with the peptide RPRAATF (SEQ ID NO:9) [45] as described previously [20].

Please replace the paragraph at page 82, lines 12-17 with the amended paragraph as follows:

An assay is set up with PDK1, GST-PKBα and the PKBα substrate, RPRAATF (SEQ ID NO:9), (as described in Example 1) but with no 3-phosphoinositide. No activation of PKBα is observed. Compounds are tested in the assay and those that give rise to activation of PKBα via PDK1 are selected for further study. Phosphatidylinositol-3,4,5-triphosphate is used as a positive control.